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PATENT
Docket No. P-4423

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): D. J. Wright, M. A. Milla, J. G. Nadeau and G. T. Walker

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TITLE: Methods and Oligonucleotides for Detecting Nucleic Acid Sequence Variations

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231
On: February 2, 2001
By: Donna M. Baumann
Donna M Baumann 2-2-01
(signature) (date)

Assistant Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

DECLARATION UNDER 37 CFR §1.132

I, James G. Nadeau, hereby declare as follows:

I am an inventor of the invention disclosed and claimed in the subject patent application.

I have read and understand the Office Action mailed September 20, 2000, finally rejecting Claims 1-24 and 55-62 of the application. I have also reviewed the references relied upon in that rejection. My opinion of the teachings of the references and their relevance to the final rejection is as follows.

Schram et al. (US Patent No. 5,681,705) describe certain amplification primers which are used for complex-specific amplification of target sequences in species of the *Mycobacterium avium* Complex. These primer sequences are set forth as SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3 in Table 1 of Schram et al. SEQ ID NOs:1 and 3 hybridize to the *M. avium* target without mismatches and to the *M. intracellulare* target with one mismatch in each of the primers. SEQ ID NO:2 hybridizes to the *M. intracellulare* target without mismatches and to the *M. avium* target with a single mismatch. The Examiner asserts that each of these primers comprise a diagnostic nucleotide for a single nucleotide difference between *M. avium* and *M. intracellulare* which is the 3' terminal nucleotide of the primer. The accompanying Figure shows that this assertion is incorrect. The Figure illustrates the hybridization of SEQ ID NO:1 (Prim1) and SEQ ID NO:2 (IN2B) of Schram et al. to their target sequences in *M. avium* and

M. intracellulare. The *M. avium* and *M. intracellulare* sequencs shown are found in S. I. Takewaki, et al. 1994. *Int. J. Syst. Bacteriol.* 44, 159-166. The single nucleotide mismatches, i.e., the diagnostic nucleotides of the primers, are bolded and underlined. It is clear that the mismatch of SEQ ID NO:2 with the *M. avium* target is at the 5' end of the target binding sequence of the primer, twelve nucleotides from the 3' terminus. Similarly, the mismatch of SEQ ID NO:1 with the *M. intracellulare* target is six nucleotides from the 3' terminus of the primer. Not shown in the Figure is hybridization of SEQ ID NO:3 (Prim2) of Schram et al., which has a target binding sequence which differs from SEQ ID NO:2 only by a C instead of a G at the 5' end, resulting in a -12 mismatch with *M. intracellulare*. It is my understanding that Claim 1 of the subject patent application recites a diagnostic nucleotide which is at the 3' terminus of the primer or within about one to four nucleotides from the 3' terminus. The three amplification primers disclosed by Schram et al. do not meet the claimed criteria for placement of the diagnostic nucleotide.

Example 4 of Schram et al. describes the performance of SEQ ID NO:1-3 in amplification reactions for detection of *M. avium* and *M. intracellulare*. Beginning at Col. 10, Ln. 49 it is disclosed that the SEQ ID NO:1/SEQ ID NO:3 amplification pair amplified the *M. avium* target 50-fold more efficiently than the *M. intracellulare* target. This primer pair is perfectly complementary to the *M. avium* target and contains a single mismatch in each primer when hybridized to the *M. intracellulare* target, i.e., there are two mismatches when amplifying *M. intracellulare* (one at -6 of one primer and one at -12 of the other primer). This double-mismatch, double-detector primer system reportedly results in a 50-fold difference in amplification efficiency between the two species in a Strand Displacement Amplification system.

Example 4 also reports the results of Strand Displacement Amplification when SEQ ID NO:2 (*M. intracellulare*-specific) is substituted for SEQ ID NO:3 (*M. avium*-specific). This substitution results in an amplification system where there is now a single primer containing mismatch with the *M. avium* target. At Col. 10, Lns. 63-67 Schram et al. report that in spite of the mismatch this amplification system unexpectedly did not result in any reduction in amplification efficiency for *M. avium*. Therefore, the SEQ ID NO:1/SEQ ID NO:2 primer pair was inadequate to discriminate between *M. avium* and *M. intracellulare*.

In making the present invention, it was discovered that proper placement of the diagnostic nucleotide is essential to achieve discrimination between targets when using a single detector primer in a strand displacement detection reaction. In making this discovery, we made four target oligonucleotides and at a defined position in each sequence we placed one of the four possible nucleotides (A, C, G or T). We then synthesized a set of seven detector primers that would hybridize to the polymorphic target with the diagnostic nucleotide at different positions within the primer. The primer set included diagnostic

nucleotides at positions from -1 to -7. In this experiment all detector primers had T as the diagnostic nucleotide, which should be specific for the A target. We found that the specificity of the T detector primer for the A target was excellent when the diagnostic nucleotide was one or two bases 5' of the detector primer's 3' nucleotide. Recessing the diagnostic nucleotide further from the 3' end resulted in degradation of specificity. By the time the diagnostic nucleotide reached the -5 position the signal from the incorrectly matched G target was nearly equal to the signal from the correctly matched A target.

Therefore, whereas Schram et al. were unsuccessful, we found that by moving the diagnostic nucleotide to the 3' terminus of the target binding sequence or to within about one to four nucleotides from the 3' terminus we were able to discriminate between single nucleotide polymorphisms using a single detector primer. Our results show that recessing the diagnostic nucleotide further from the 3' end of the target binding sequence results in loss of specificity and an inability to discriminate between alleles. This was unexpected because, based on the known effects of nucleotide mismatch position on the melting temperature of DNA hybrids, moving the diagnostic nucleotide toward the center of the target binding region would be expected to be more destabilizing for a mismatched primer than a mismatch near the terminus. One would therefore expect to observe increasing specificity for the correctly matched target as the diagnostic nucleotide is moved toward the center of the target binding sequence. Unexpectedly, in our strand displacing detection reaction we observed the opposite effect. It is my opinion that, based on the teachings of Schram et al., one would not predict the presently claimed positioning of the diagnostic nucleotide to achieve discrimination of single nucleotide differences using a single detector primer in a strand displacing reaction.

Vary et al. describe a primer with a 3' terminal mismatch for detection of sequence variations, but do not suggest use of such a primer in a strand displacing reaction. As discussed above, Schram et al. teach internal primer mismatches but fail to obtain nucleotide discrimination using a single mismatched primer. Neither reference in any way suggests that the 3' terminal mismatch of Vary et al. might solve the problem encountered by Schram et al. or that a mismatch at about -1 to -4 would also be successful, especially in view of the fact that placing the mismatch at or near the 3' terminus in a strand displacing detection reaction is counterintuitive.

In conclusion, it is my belief that the references cited against the claims of the subject patent application do not teach or suggest the claimed strand displacing detection system employing a single detector primer containing a diagnostic nucleotide which is at the 3' terminus or within 1-4 nucleotides from the 3' terminus.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

James G Nadeau

James G. Nadeau

1/30/2001

Date

*dna*J Sequence Alignments for M. tuberculosis (Mtb), M. intracellulare (M.in), & M. avium (M.av.)

(Alignments taken from Takewaki *et al* (1994) *Int. J. Sys. Bacteriol.* ***44***, 159-166.)

Bold underlined nucleotides denote a mismatch between the primer and either the M.in. or M. av target.